1 TCCGGGGGCC ATCATCATCA TCATCATAGC TCCGGAGACG ATGATGACAA GATGAGCTAC

1▸SerGlyGlyH isHisHisHi sHisHisSer SerGlyAspA spAspAspLy sMetSerTyr

61 AACTTGCTTG GATTCCTACA AAGAAGCAGC AATTTTCAGT GTCAGAAGCT CCTGTGGCAA

21▸AsnLeuLeuG lyPheLeuGl nArgSerSer AsnPheGlnC ysGlnLysLe uLeuTrpGln

121 TTGAATGGGA GGCTTGAATA CTGCCTCAAG GACAGGATGA ACTTTGACAT CCCTGAGGAG

41▸LeuAsnGlyA rgLeuGluTy rCysLeuLys AspArgMetA snPheAspIl eProGluGlu

181 ATTAAGCAGC TGCAGCAGTT CCAGAAGGAG GACGCCGCAT TGACCATCTA TGAGATGCTC

61▸IleLysGlnL euGlnGlnPh eGlnLysGlu AspAlaAlaL euThrIleTy rGluMetLeu

241 CAGAACATCT TTGCTATTTT CAGACAAGAT TCATCTAGCA CTGGCTGGAA TGAGACTATT

81▸GlnAsnIleP heAlaIlePh eArgGlnAsp SerSerSerT hrGlyTrpAs nGluThrIle

301 GTTGAGAACC TCCTGGCTAA TGTCTATCAT CAGATAAACC ATCTGAAGAC AGTCCTGGAA

101▸ValGluAsnL euLeuAlaAs nValTyrHis GlnIleAsnH isLeuLysTh rValLeuGlu

361 GAAAAACTGG AGAAAGAAGA TTTCACCAGG GGAAAACTCA TGAGCAGTCT GCACCTGAAA

121▸GluLysLeuG luLysGluAs pPheThrArg GlyLysLeuM etSerSerLe uHisLeuLys

421 AGATATTATG GGAGGATTCT GCATTACCTG AAGGCCAAGG AGTACAGTCA CTGTGCCTGG

141▸ArgTyrTyrG lyArgIleLe uHisTyrLeu LysAlaLysG luTyrSerHi sCysAlaTrp

481 ACCATAGTCA GAGTGGAAAT CCTAAGGAAC TTTTACTTCA TTAACAGACT TACAGGTTAC

161▸ThrIleValA rgValGluIl eLeuArgAsn PheTyrPheI leAsnArgLe uThrGlyTyr

541 CTCCGAAAC

181▸LeuArgAsn

FIG. 1

FIG. 2A-1
FIG. 2A-2

FIG. 2A

FIG. 2A-1

1 ATGAGCTACA ACTTGCTTGG ATTCCTACAA AGAAGCAGCA ATTTTCAGTG TCAGAAGCTC

1▸MetSerTyrA snLeuLeuGl yPheLeuGln ArgSerSerA snPheGlnCy sGlnLysLeu

61 CTGTGGCAAT TGAATGGGAG GCTTGAATAC TGCCTCAAGG ACAGGATGAA CTTTGACATC

21▸LeuTrpGlnL euAsnGlyAr gLeuGluTyr CysLeuLysA spArgMetAs nPheAspIle

121 CCTGAGGAGA TTAAGCAGCT GCAGCAGTTC CAGAAGGAGG ACGCCGCATT GACCATCTAT

41▸ProGluGluI leLysGlnLe uGlnGlnPhe GlnLysGluA spAlaAlaLe uThrIleTyr

181 GAGATGCTCC AGAACATCTT TGCTATTTTC AGACAAGATT CATCTAGCAC TGGCTGGAAT

61▸GluMetLeuG lnAsnIlePh eAlaIlePhe ArgGlnAspS erSerSerTh rGlyTrpAsn

241 GAGACTATTG TTGAGAACCT CCTGGCTAAT GTCTATCATC AGATAAACCA TCTGAAGACA

81▸GluThrIleV alGluAsnLe uLeuAlaAsn ValTyrHisG lnIleAsnHi sLeuLysThr

301 GTCCTGGAAG AAAAACTGGA GAAAGAAGAT TTCACCAGGG GAAAACTCAT GAGCAGTCTG

101▸ValLeuGluG luLysLeuGl uLysGluAsp PheThrArgG lyLysLeuMe tSerSerLeu

361 CACCTGAAAA GATATTATGG GAGGATTCTG CATTACCTGA AGGCCAAGGA GTACAGTCAC

121▸HisLeuLysA rgTyrTyrGl yArgIleLeu HisTyrLeuL ysAlaLysGl uTyrSerHis

421 TGTGCCTGGA CCATAGTCAG AGTGGAAATC CTAAGGAACT TTTACTTCAT TAACAGACTT

141▸CysAlaTrpT hrIleValAr gValGluIle LeuArgAsnP heTyrPheIl eAsnArgLeu

481 ACAGGTTACC TCCGAAACGA CGATGATGAC AAGGTCGACA AAACTCACAC ATGCCCACCG

161▸ThrGlyTyrL euArgAsnAs pAspAspAsp LysValAspL ysThrHisTh rCysProPro

541 TGCCCAGCAC CTGAACTCCT GGGGGGACCG TCAGTCTTCC TCTTCCCCCC AAAACCCAAG

FIG. 2A-2

181 ▸CysProAlaP roGluLeuLe uGlyGlyPro SerValPheL euPheProPr oLysProLys

601 GACACCCTCA TGATCTCCCG GACCCCTGAG GTCACATGCG TGGTGGTGGA CGTGAGCCAC

201 ▸AspThrLeuM etIleSerAr gThrProGlu ValThrCysV alValValAs pValSerHis

661 GAAGACCCTG AGGTCAAGTT CAACTGGTAC GTGGACGGCG TGGAGGTGCA TAATGCCAAG

221 ▸GluAspProG luValLysPh eAsnTrpTyr ValAspGlyV alGluValHi sAsnAlaLys

FIG. 2B

721 ACAAAGCCGC GGGAGGAGCA GTACAACAGC ACGTACCGTG TGGTCAGCGT CCTCACCGTC

1 ▸ThrLysProA rgGluGluGl nTyrAsnSer ThrTyrArgV alValSerVa lLeuThrVal

781 CTGCACCAGG ACTGGCTGAA TGGCAAGGAG TACAAGTGCA AGGTCTCCAA CAAAGCCCTC

21 ▸LeuHisGlnA spTrpLeuAs nGlyLysGlu TyrLysCysL ysValSerAs nLysAlaLeu

841 CCAGCCCCCA TCGAGAAAAC CATCTCCAAA GCCAAAGGGC AGCCCCGAGA ACCACAGGTG

41 ▸ProAlaProI leGluLysTh rIleSerLys AlaLysGlyG lnProArgGl uProGlnVal

901 TACACCCTGC CCCCATCCCG GGATGAGCTG ACCAAGAACC AGGTCAGCCT GACCTGCCTG

61 ▸TyrThrLeuP roProSerAr gAspGluLeu ThrLysAsnG lnValSerLe uThrCysLeu

961 GTCAAAGGCT TCTATCCCAG CGACATCGCC GTGGAGTGGG AGAGCAATGG GCAGCCGGAG

81 ▸ValLysGlyP heTyrProSe rAspIleAla ValGluTrpG luSerAsnGl yGlnProGlu

1021 AACAACTACA AGACCACGCC TCCCGTGTTG GACTCCGACG GCTCCTTCTT CCTCTACAGC

101 ▸AsnAsnTyrL ysThrThrPr oProValLeu AspSerAspG lySerPhePh eLeuTyrSer

1081 AAGCTCACCG TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG CTCCGTGATG

121 ▸LysLeuThrV alAspLysSe rArgTrpGln GlnGlyAsnV alPheSerCy sSerValMet

1141 CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCCTCT CCCTGTCTCC CGGGAAA

141 ▸HisGluAlaL euHisAsnHi sTyrThrGln LysSerLeuS erLeuSerPr oGlyLys

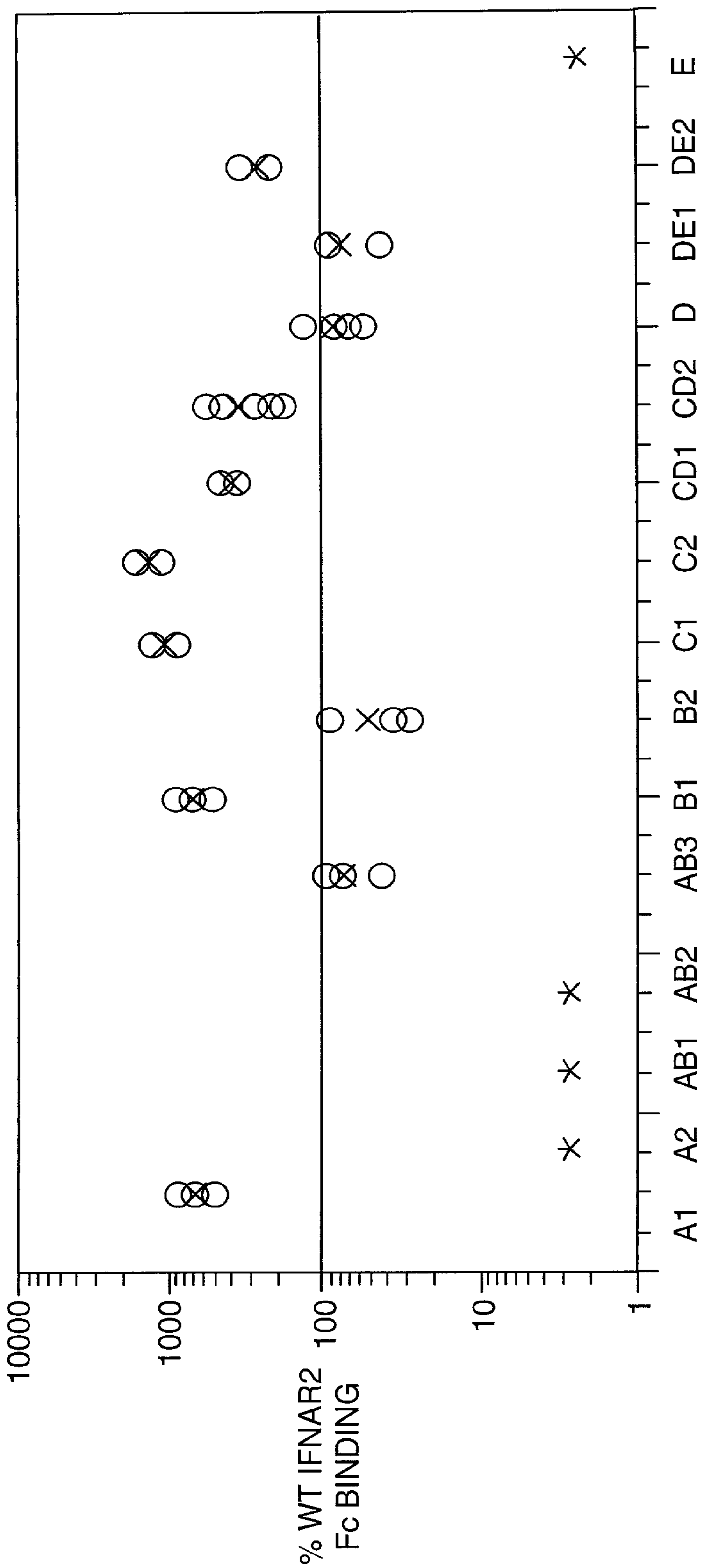

% WT IFNAR2
Fc BINDING
10000
1000
100
10
1
A1
A2
AB1
AB2
AB3
B1
B2
C1
C2
CD1
CD2
D
DE1
DE2
E

FIG. 3

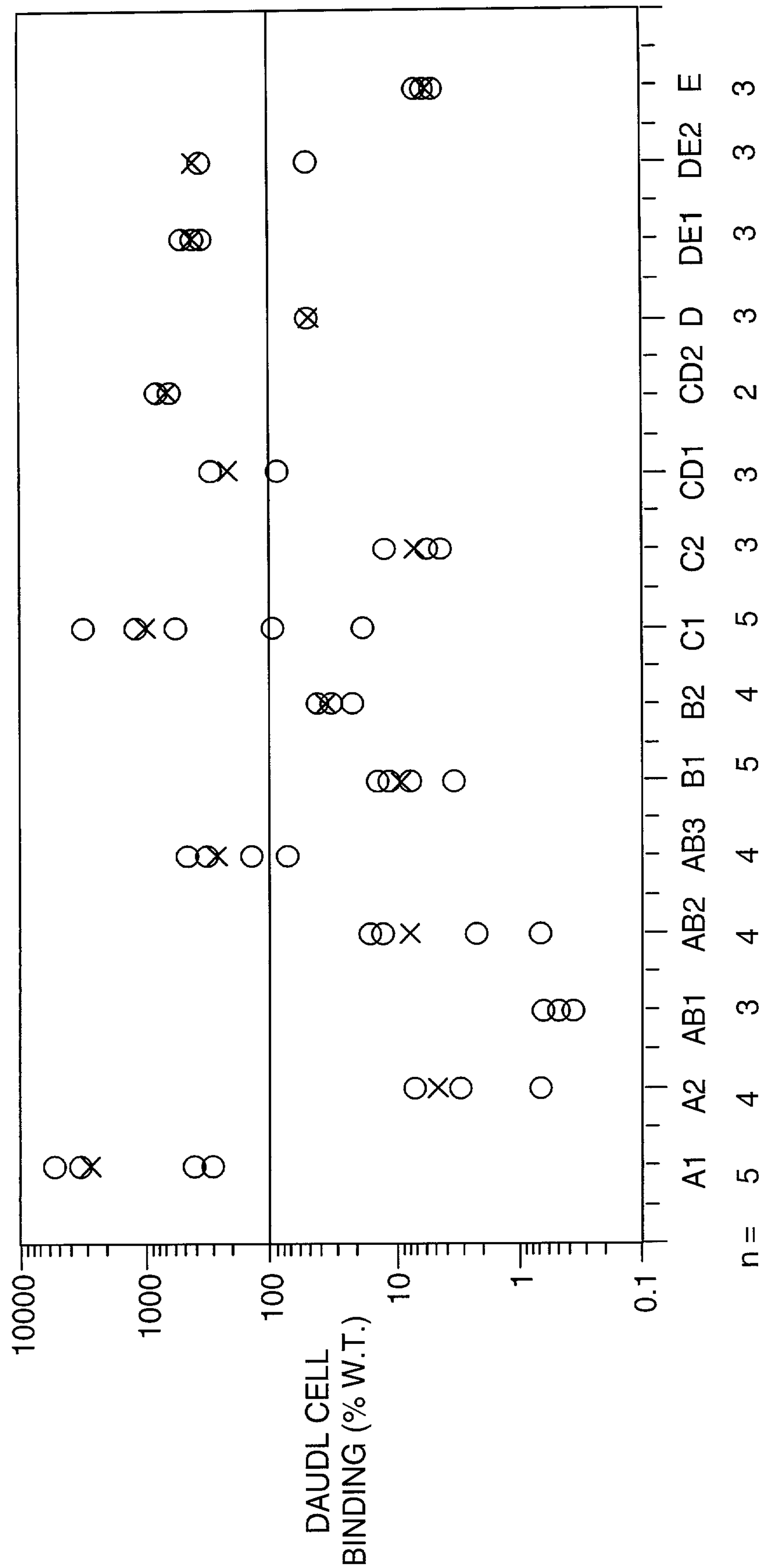

DAUDL CELL BINDING (% W.T.)
10000
1000
100
10
1
0.1
A1 A2 AB1 AB2 AB3 B1 B2 C1 C2 CD1 CD2 D DE1 DE2 E
n = 5 4 3 4 4 5 4 5 3 3 2 3 3 3 3

FIG. 4

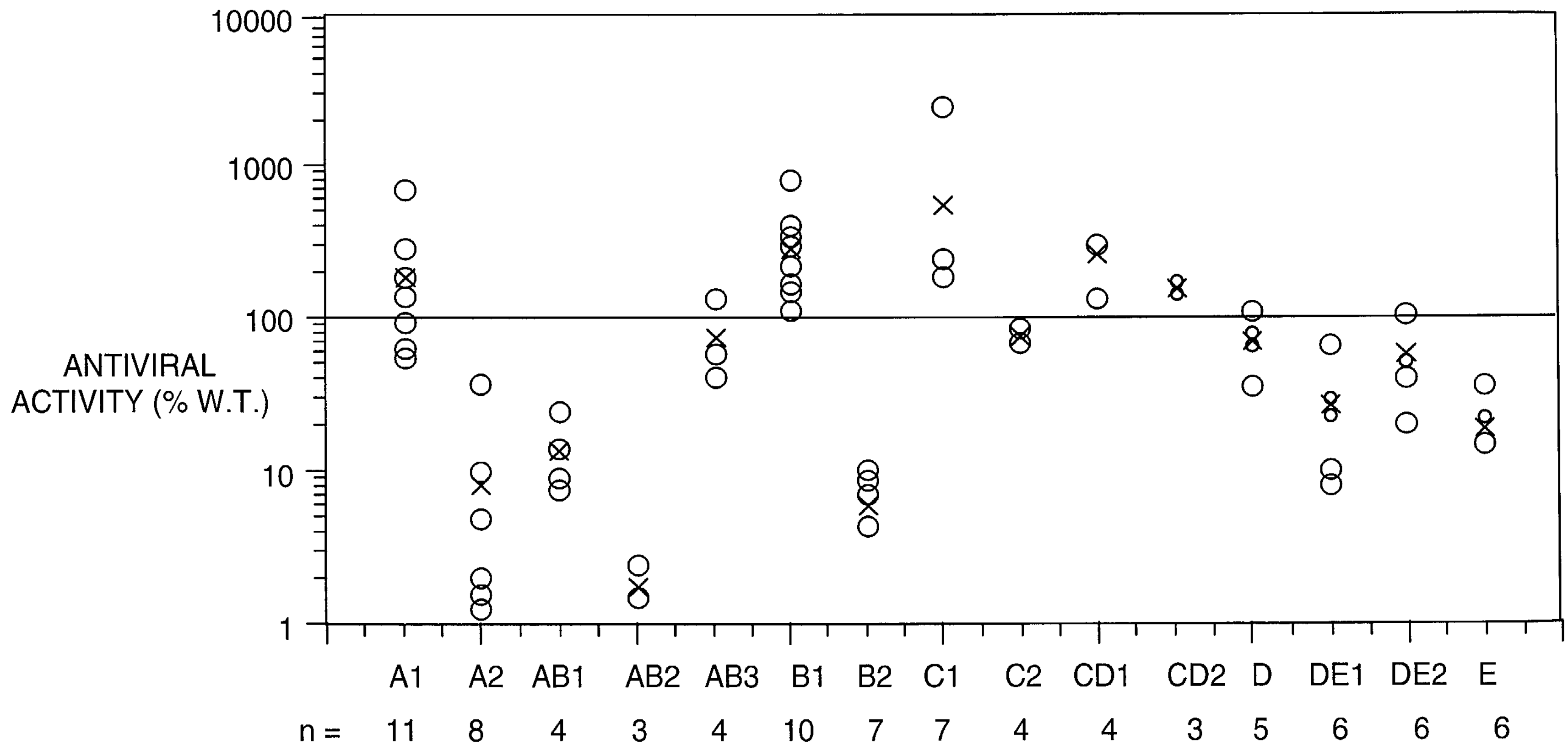

10000
1000
100
10
1
ANTIVIRAL
ACTIVITY (% W.T.)
A1 A2 AB1 AB2 AB3 B1 B2 C1 C2 CD1 CD2 D DE1 DE2 E
n = 11 8 4 3 4 10 7 7 4 4 3 5 6 6 6

FIG. 5

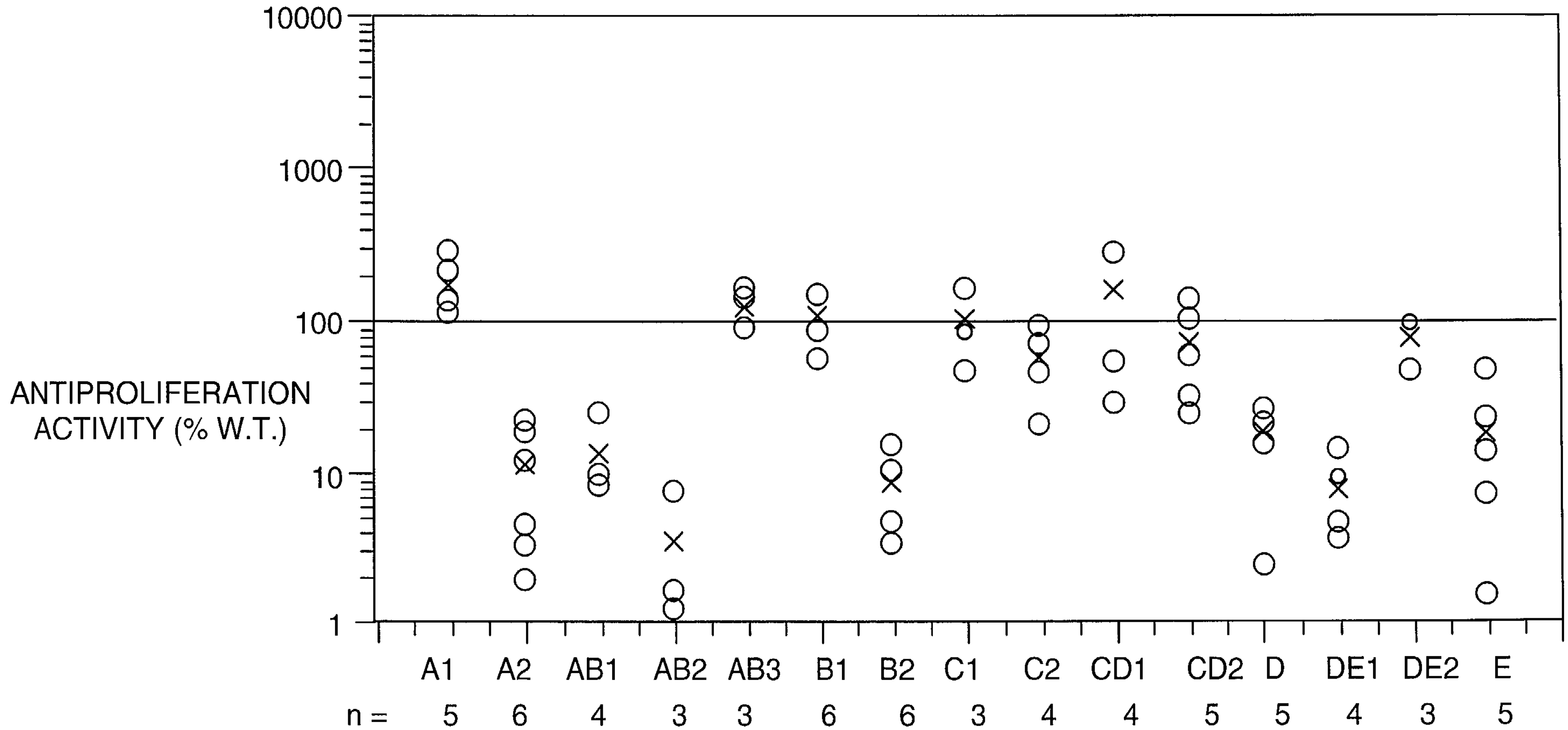
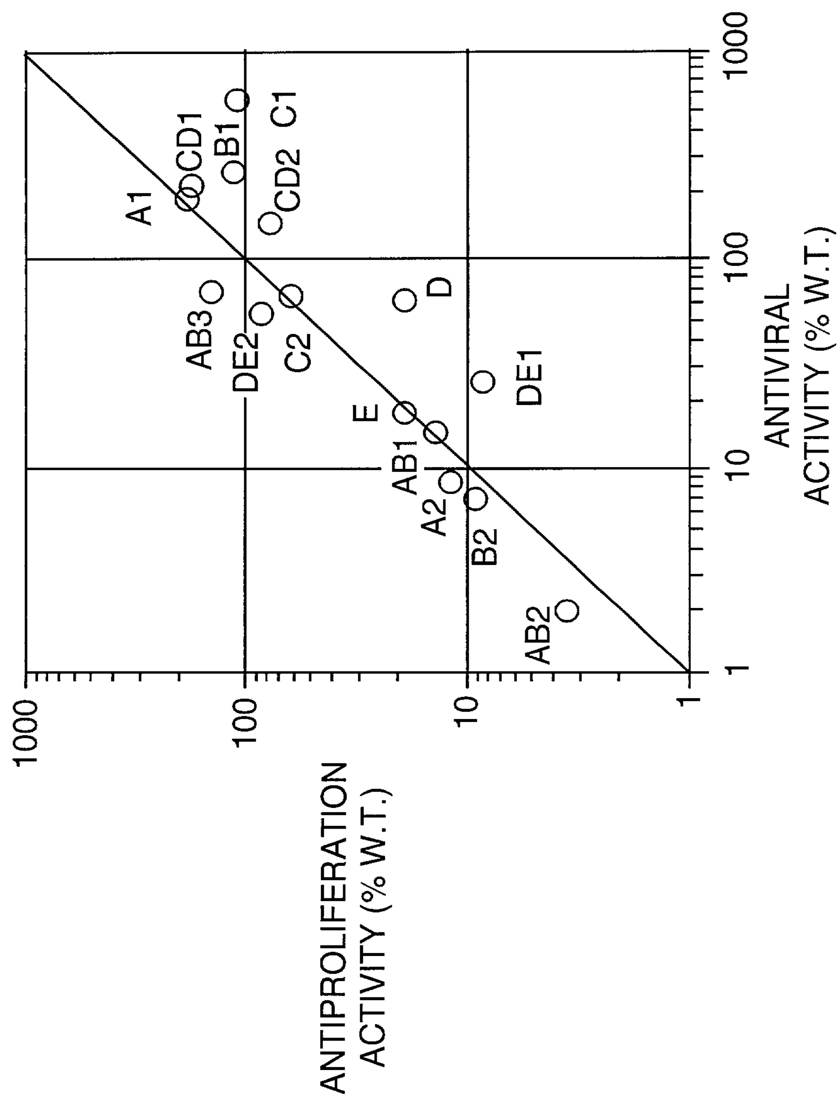
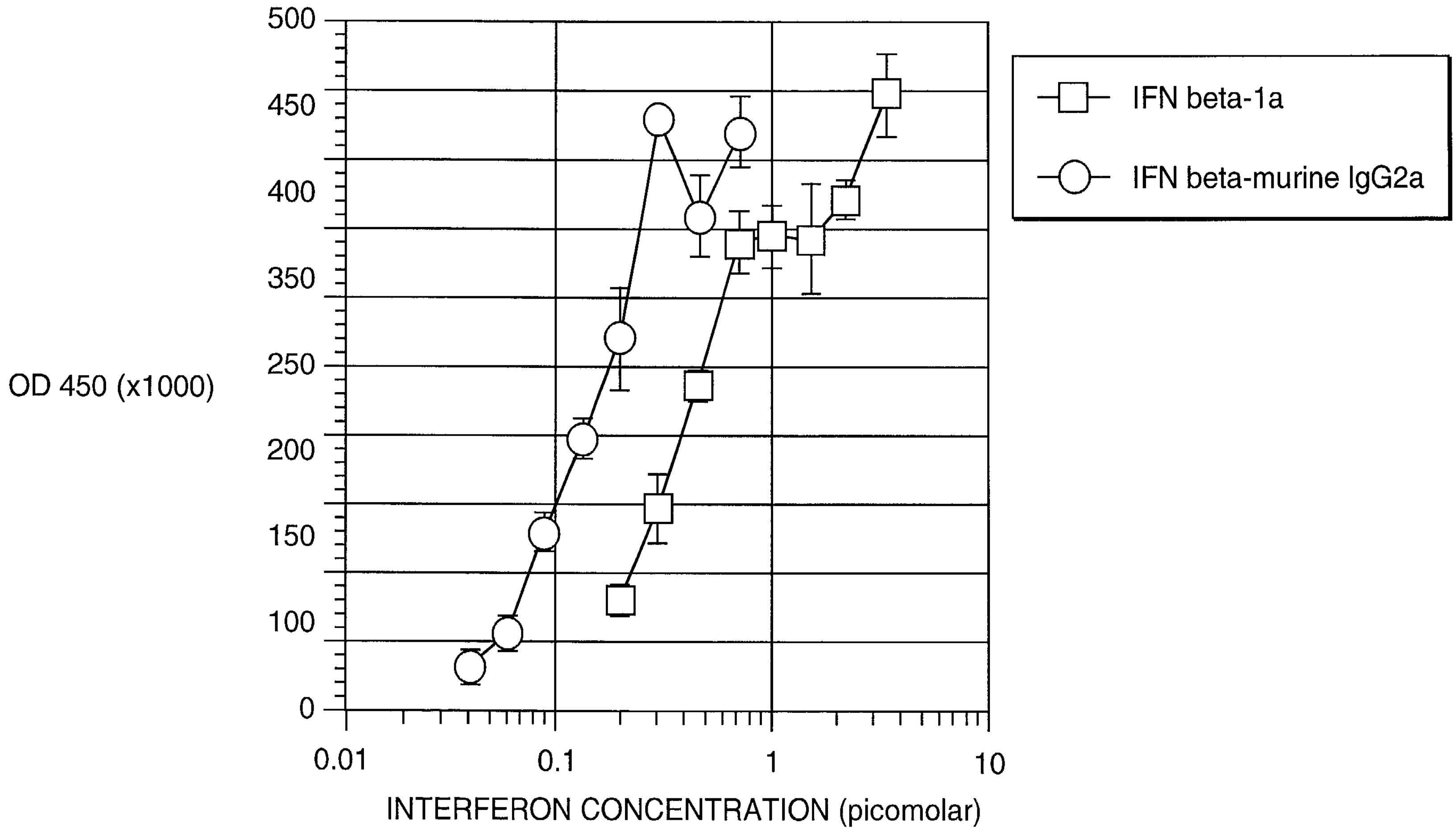

ANTIPROLIFERATION ACTIVITY (% W.T.)
10000
1000
100
10
1
A1 A2 AB1 AB2 AB3 B1 B2 C1 C2 CD1 CD2 D DE1 DE2 E
n = 5 6 4 3 3 6 6 3 4 4 5 5 4 3 5

FIG. 6



ANTIPROLIFERATION ACTIVITY (% W.T.)
1000
100
10
1
ANTIVIRAL ACTIVITY (% W.T.)
1
10
100
1000
A1
CD1
B1
C1
CD2
AB3
DE2
C2
D
E
DE1
AB1
A2
B2
AB2

FIG. 7



ANTIVIRAL ACTIVITY OF IFN beta-murine-IgG2a FUSION PROTEIN

FIG. 8

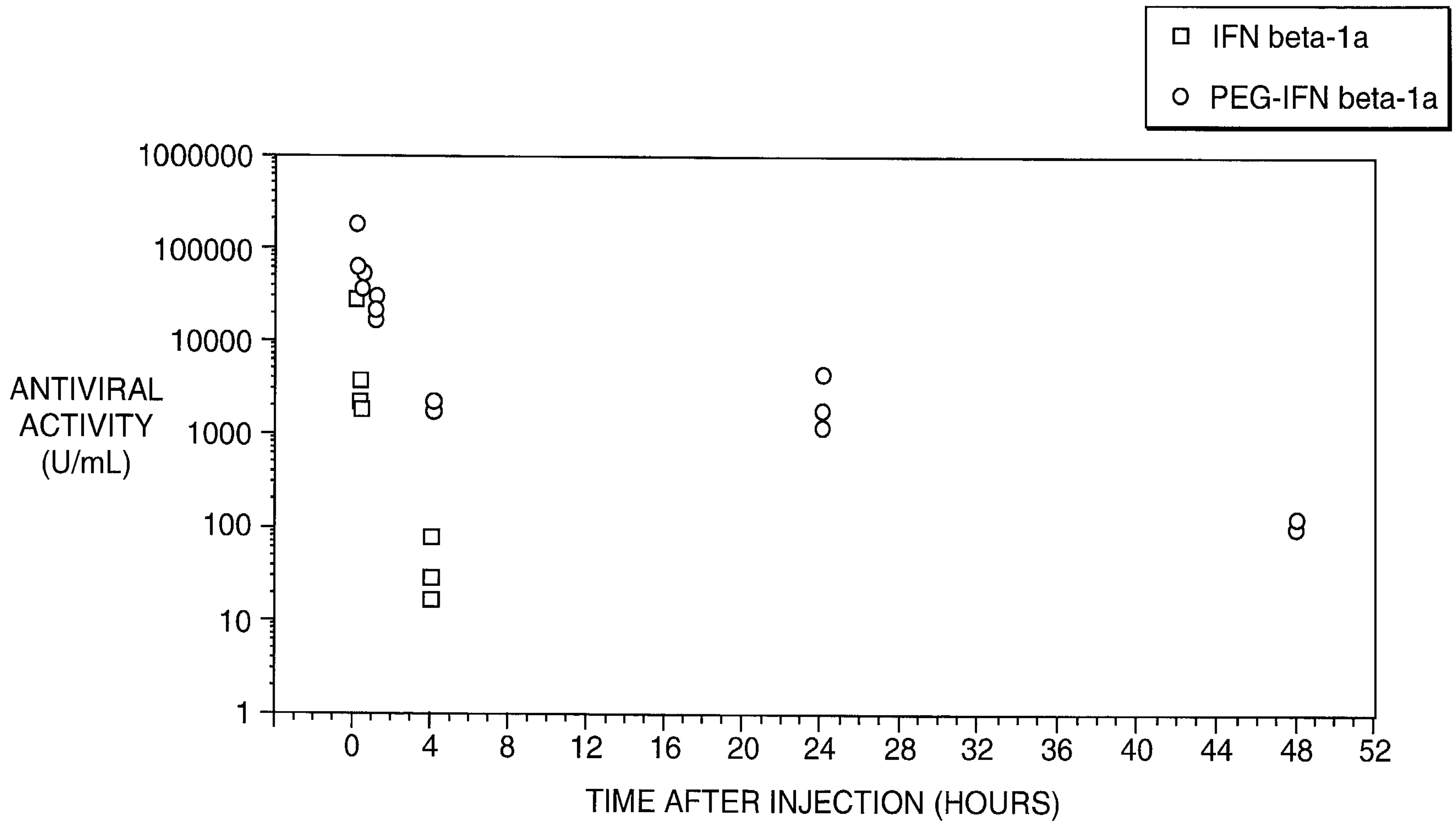

IFN beta-1a
PEG-IFN beta-1a
ANTIVIRAL
ACTIVITY
(U/mL)
1000000
100000
10000
1000
100
10
1
0
4
8
12
16
20
24
28
32
36
40
44
48
52
TIME AFTER INJECTION (HOURS)

FIG. 9

FIG. 10A
FIG. 10B
FIG. 10C

FIG. 10

IFNβ G162C-Ig direct fusion construct open reading frame

```
  1 ATGCCTGGGAAGATGGTCGTGATCCTTGGAGCCTCAAATATACTTTGGATAATGTTTGCA 60
    M  P  G  K  M  V  V  I  L  G  A  S  N  I  L  W  I  M  F  A

 61 GCTTCTCAAGCCATGAGCTACAACTTGCTTGGATTCCTACAAAGAAGCAGCAATTTTCAG 120
    A  S  Q  A  M  S  Y  N  L  L  G  F  L  Q  R  S  S  N  F  Q

121 TGTCAGAAGCTCCTGTGGCAATTGAATGGGAGGCTTGAATACTGCCTCAAGGACAGGATG 180
    C  Q  K  L  L  W  Q  L  N  G  R  L  E  Y  C  L  K  D  R  M

181 AACTTTGACATCCCTGAGGAGATTAAGCAGCTGCAGCAGTTCCAGAAGGAGGACGCCGCA 240
    N  F  D  I  P  E  E  I  K  Q  L  Q  Q  F  Q  K  E  D  A  A

241 TTGACCATCTATGAGATGCTCCAGAACATCTTTGCTATTTTCAGACAAGATTCATCTAGC 300
    L  T  I  Y  E  M  L  Q  N  I  F  A  I  F  R  Q  D  S  S  S

301 ACTGGCTGGAATGAGACTATTGTTGAGAACCTCCTGGCTAATGTCTATCATCAGATAAAC 360
    T  G  W  N  E  T  I  V  E  N  L  L  A  N  V  Y  H  Q  I  N

361 CATCTGAAGACAGTCCTGGAAGAAAAACTGGAGAAAGAAGATTTCACCAGGGGAAAACTC 420
    H  L  K  T  V  L  E  E  K  L  E  K  E  D  F  T  R  G  K  L

421 ATGAGCAGTCTGCACCTGAAAAGATATTATGGGAGGATTCTGCATTACCTGAAGGCCAAG 480
    M  S  S  L  H  L  K  R  Y  Y  G  R  I  L  H  Y  L  K  A  K
```

FIG. 10A

```
481 GAGTACAGTCACTGTGCCTGGACCATAGTCAGAGTGGAAATCCTAAGGAACTTTTACTTC 540
    E  Y  S  H  C  A  W  T  I  V  R  V  E  I  L  R  N  F  Y  F

541 ATTAACAGACTTACATGTTACCTCCGAAACGTCGACAAAACTCACACATGCCCACCGTGC 600
    I  N  R  L  T  C  Y  L  R  N  V  D  K  T  H  T  C  P  P  C

601 CCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGAC 660
    P  A  P  E  L  L  G  G  P  S  V  F  L  F  P  P  K  P  K  D

661 ACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA 720
    T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S  H  E

721 GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACA 780
    D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A  K  T

781 AAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTG 840
    K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L

841 CACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA 900
    H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P

901 GCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTAC 960
    A  P  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y
```

FIG. 10B

```
961  ACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC 1020
     T  L  P  P  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V

1021 AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC 1080
     K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N

1081 AACTACAAGACCACGCCTCCCGTGTTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAG 1140
    N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K

1141 CTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT 1200
     L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V  M  H

1201 GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCCGGGAAATGA    1257
     E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  P  G  K  *
```

FIG. 10C

FIG. 11A
FIG. 11B
FIG. 11C

FIG. 11

IFNβ G162C-Ig fusion G4S linker construct open reading frame

```
  1 ATGCCTGGGAAGATGGTCGTGATCCTTGGAGCCTCAAATATACTTTGGATAATGTTTGCA  60
    M  P  G  K  M  V  V  I  L  G  A  S  N  I  L  W  I  M  F  A

 61 GCTTCTCAAGCCATGAGCTACAACTTGCTTGGATTCCTACAAAGAAGCAGCAATTTTCAG 120
    A  S  Q  A  M  S  Y  N  L  L  G  F  L  Q  R  S  S  N  F  Q

121 TGTCAGAAGCTCCTGTGGCAATTGAATGGGAGGCTTGAATACTGCCTCAAGGACAGGATG 180
    C  Q  K  L  L  W  Q  L  N  G  R  L  E  Y  C  L  K  D  R  M

181 AACTTTGACATCCCTGAGGAGATTAAGCAGCTGCAGCAGTTCCAGAAGGAGGACGCCGCA 240
    N  F  D  I  P  E  E  I  K  Q  L  Q  Q  F  Q  K  E  D  A  A

241 TTGACCATCTATGAGATGCTCCAGAACATCTTTGCTATTTTCAGACAAGATTCATCTAGC 300
    L  T  I  Y  E  M  L  Q  N  I  F  A  I  F  R  Q  D  S  S  S

301 ACTGGCTGGAATGAGACTATTGTTGAGAACCTCCTGGCTAATGTCTATCATCAGATAAAC 360
    T  G  W  N  E  T  I  V  E  N  L  L  A  N  V  Y  H  Q  I  N

361 CATCTGAAGACAGTCCTGGAAGAAAAACTGGAGAAAGAAGATTTCACCAGGGGAAAACTC 420
    H  L  K  T  V  L  E  E  K  L  E  K  E  D  F  T  R  G  K  L
```

FIG. 11A

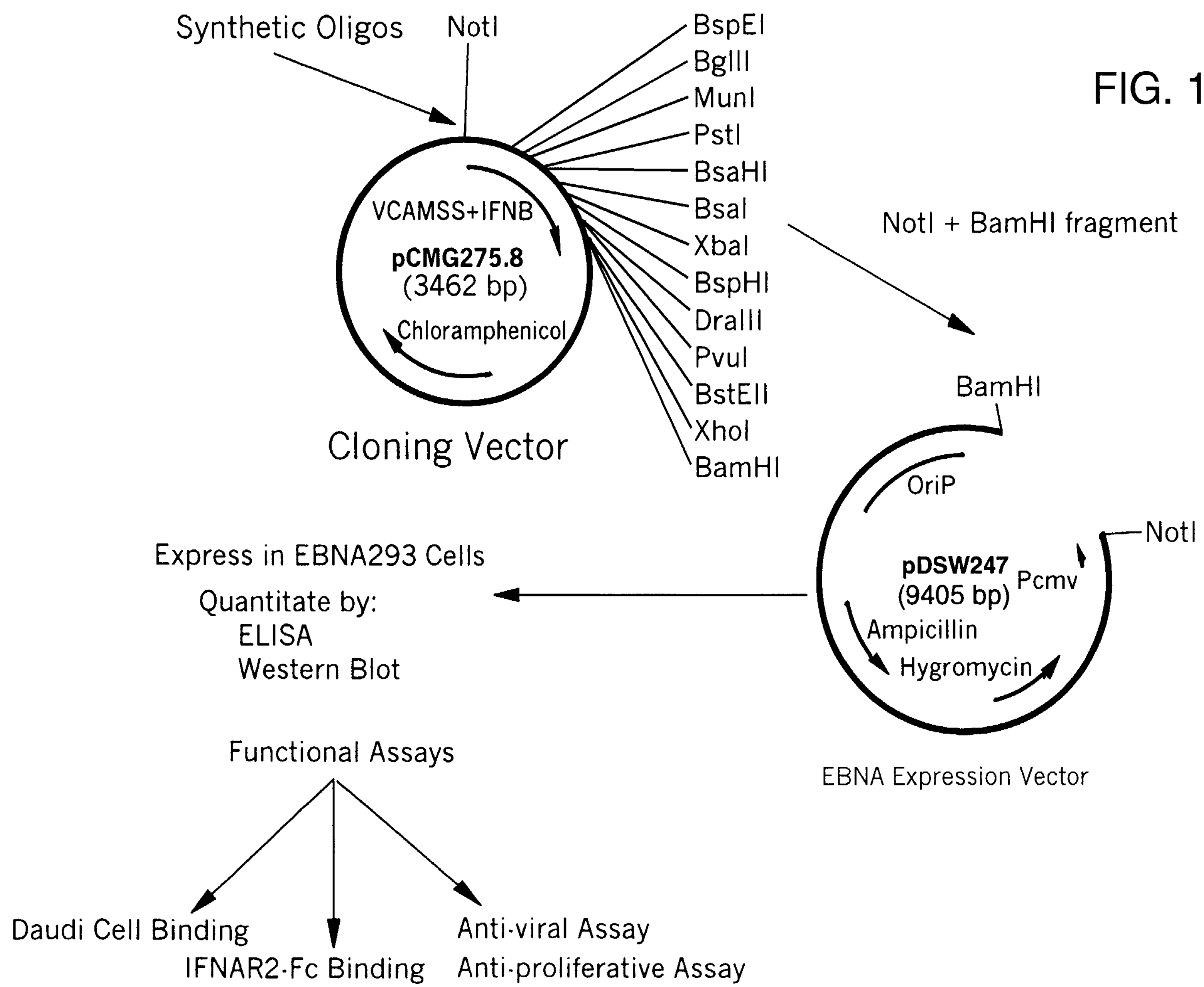
```
421 ATGAGCAGTCTGCACCTGAAAAGATATTATGGGAGGATTCTGCATTACCTGAAGGCCAAG 480
    M  S  S  L  H  L  K  R  Y  Y  G  R  I  L  H  Y  L  K  A  K

481 GAGTACAGTCACTGTGCCTGGACCATAGTCAGAGTGGAAATCCTAAGGAACTTTTACTTC 540
    E  Y  S  H  C  A  W  T  I  V  R  V  E  I  L  R  N  F  Y  F

541 ATTAACAGACTTACATGTTACCTCCGAAACGGCGGTGGTGGCAGCGTCGACAAAACTCAC 600
    I  N  R  L  T  C  Y  L  R  N  G  G  G  G  S  V  D  K  T  H

601 ACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCC 660
    T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F  P

661 CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTG 720
    P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V

721 GACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTG 780
    D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V

781 CATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGC 840

    H  N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S

841 GTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC 900
    V  L  T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S

901 AACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA 960
    N  K  A  L  P  A  P  I  E  K  T  I  S  K  A  K  G  Q  P  R
```

FIG. 11B

```
961  GAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGC 1020
     E  P  Q  V  Y  T  L  P  P  S  R  D  E  L  T  K  N  Q  V  S

1021 CTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT 1080
     L  T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N

1081 GGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGTTGGACTCCGACGGCTCCTTC 1140
     G  Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F

1141 TTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCA 1200
     F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S

1201 TGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCT 1260
     C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S

1261 CCCGGGAAATGA 1272
     P  G  K  *
```

FIG. 11C

FIG. 12


Synthetic Oligos
NotI
BspEI
BglII
MunI
PstI
BsaHI
BsaI
XbaI
BspHI
DraIII
PvuI
BstEII
XhoI
BamHI
VCAMSS+IFNB
pCMG275.8
(3462 bp)
Chloramphenicol
Cloning Vector
NotI + BamHI fragment
BamHI
OriP
NotI
pDSW247
(9405 bp)
Pcmv
Ampicillin
Hygromycin
EBNA Expression Vector
Express in EBNA293 Cells
Quantitate by:
ELISA
Western Blot
Functional Assays
Daudi Cell Binding
IFNAR2-Fc Binding
Anti-viral Assay
Anti-proliferative Assay